

## *In-Situ X-Ray Microscopy Reveals Impact of Environmental Context on Structure*

**F**ormed by the biochemical transformation of plant and animal residue, humic substances (HSs) constitute a major fraction of the dissolved and particulate organic matter in natural ecosystems. The ubiquitous presence of HSs, combined with their ability to provide multiple sites for chemical reaction, makes them relevant to numerous biogeochemical processes such as mineral weathering, nutrient bioavailability, and contaminant transport. The reactivity of HSs depends on their functional group chemistry and microstructure, which are in turn influenced by the composition of the surrounding media. Researchers from Princeton University, the University of Puerto Rico, and Berkeley Lab have used the high-resolution x-ray microscope at Beamline 6.1.2 to perform *in-situ* studies of how changes in surrounding media affect the structure of HSs.

In the ALS x-ray microscope, image contrast stems from the photoelectric absorption of x rays, and hence, element- and functional-

group-specific images can be obtained at high spectral resolution for organic molecules. Although significant information on the structure of HSs has been obtained previously by using electron microscopy, nuclear magnetic resonance, and infrared spectroscopy, until now, direct evidence for how structural transformations depend on the sample's origin (soil vs. water), solution chemistry, and substrate mineralogy has not been documented. Furthermore, because of the structural changes associated with sample drying, the structures obtained by using techniques that require dried samples cannot be correlated with solution chemistry.

To test the influence of these parameters, the researchers conducted x-ray microscopy experiments on HSs isolated from water and soil samples. The observations were made on samples in solution under varying chemical conditions, including pH (2–12), ionic strength (0.01–2 M NaCl or CaCl<sub>2</sub>), organic molecule concentration (0.03–10 g/L carbon), and complexing cations

(1 mM Ca<sup>+2</sup> or Fe<sup>+3</sup>). Studies were also conducted in the presence of common soil minerals such as goethite, calcite, and clays (kaolinite, montmorillonite). To correlate the structures of the isolated HSs with those of undisturbed natural samples, the researchers also examined “pristine” soil organic molecules that were not extracted from the surrounding soil matrix.

The researchers found a great deal of structural variety, including sheets and globular configurations, thread- and net-like shapes, and small, uniform aggregates. The results indicate that the microstructures of humic substances are different under different solution chemical conditions, in contrast to the previously held belief that the molecules simply form rings in acidic or strong electrolyte solutions and elongate in dilute alkaline solutions. Also, HSs from soil and water require different chemical conditions to assume a particular configuration, reflecting the comparatively lower solubility, higher aromatic carbon content, and low carboxyl

content of soil materials. The presence of minerals added further complexity: the composition and thickness of HS coatings on the mineral surfaces were found to depend on pH and HS concentration and origin (soil or water). The latter observations were made possible by surface-sensitive photoemission spectroscopy methods available at the ALS. The structures observed in pristine soil samples were similar to the organomineral structures seen in the isolated soil samples, suggesting that the results obtained from the latter can be applied to HSs in soils and sediments, but not necessarily to those in solution.

The observed changes in microstructure can modify the exposed surface area and alter the functional group chemistry of the HSs, affecting, for example, protonation and cation complexation. Systematic structural studies with high-resolution *in-situ* x-ray microscopy is the first step toward understanding, predicting, and possibly controlling the chemical interactions of HSs in nature. ■

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S.C.B. Myneni, J.T. Brown, G.A. Martinez, W. Meyer-Ilse, “Imaging of Humic Substance Macromolecular Structures in Water and Soils,” *Science* **286** (1999) 1335.



# UNEARTHING THE STRUCTURE OF HUMIC SUBSTANCES



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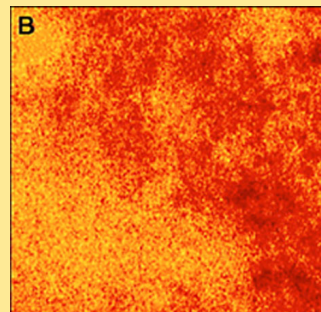
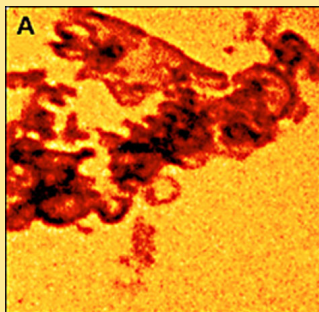
- **Humic substances: result of biochemical transformation of plant/animal residue**
  - *Relevant to numerous biogeochemical processes*
  - *Structural knowledge needed for understanding, prediction of environmental impact*
- **High-resolution in-situ x-ray microscopy studies at ALS**
  - *Element- and functional-group-specific images of organic molecules*
  - *Direct evidence of structural changes with respect to environmental context*
- **Impact of environmental context on structure**
  - *Dependence on solution chemistry more complex than previously thought*
  - *Soil- vs. water-derived humics exhibit different structures*
  - *Differences also found in composition and thickness of mineral coatings*

# UNEARTHING THE STRUCTURE OF HUMIC SUBSTANCES

## *In-Situ X-Ray Microscopy Reveals Impact of Environmental Context on Structure*

### Effect of Solution pH

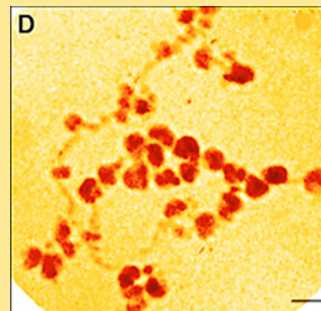
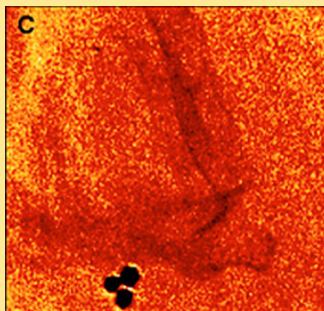
*Globular and ring-like structures in acidic solution.*



*Uniform, small aggregates in alkaline solution.*

### Effect of Cation Presence

*Thread-like structures with divalent cations.*



*Globular and thread-like structures with trivalent cations.*

Scale bar = 500 nm